A COMPARATIVE STUDY OF PLATELET COUNTS BY AUTOMATED AND MANUAL METHOD IN PATIENTS WITH THROMBOCYTOPENIA AND THROMBOCYTOSIS

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ABSTRACT

Platelet count is a vital parameter in the diagnosis and management of hematological disorders such as thrombocytopenia and thrombocytosis. Although automated hematology analyzers offer rapid and efficient platelet counting, their accuracy can be compromised in cases involving platelet clumping or morphological abnormalities, making manual methods still relevant for precise evaluation. This study aimed to evaluate the correlation and reliability of automated, Neubauer manual, and peripheral blood smear (PBS) methods for platelet counting. A hospital-based cross-sectional study was conducted over six months, including 321 patients undergoing platelet count testing, of whom 246 had thrombocytopenia and 75 had thrombocytosis. Platelet counts were measured using an automated hematology analyzer (Sysmex XN-550, Japan), Neubauer manual counting method, and PBS examination. Data were analyzed to compare and assess the correlation between the methods. The mean platelet count of thrombocytopenic cases across different methods were automated (79000± 33296/µl), manual (93900±40130/µl) and peripheral blood smear (91200±37560/µl), respectively. For thrombocytosis cases the mean platelet value across different methods were automated (597000±148000/µl), manual (590000± 164000/µl) and peripheral blood smear (601000±180000/µl), respectively. A strong correlation was observed among the methods (Neubauer manual vs automated, r=0.77, p=0.01 and Neubauer manual vs PBS r=0.78, p=0.01). There is no significant difference in platelet value between the methods. While all three methods exhibit strong agreement, the automated method may underreport platelet counts in thrombocytopenic samples. Neubauer manual and PBS methods remain valuable for confirming the low platelet counts.

KEYWORDS

Automated method, neubauer manual method, platelet count, peripheral blood smear, thrombocytopenia, thrombocytosis

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INTRODUCTION

Platelets are non-nucleated, discoid cells measuring 1–3 µm, produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes. They play crucial structural and molecular roles in blood clotting.[1] Platelet counts are frequently requested nowadays, particularly in conditions such as dengue fever, chemotherapy, bleeding disorders, pregnancy, and sepsis.[2]

Platelet count is an important component of both the diagnostic and treatment processes. Measurement of platelet counts using automated hematology analyzers is generally precise and accurate. These methods are simple, fast, and widely used in clinical practice. With the advancement of technology, hematology analyzers have evolved from semi-automated to fully automated systems, utilizing principles such as impedance, flow cytometry, and optical fluorescence. However, despite their reliability, automated analyzers may face challenges when measuring extremely low platelet counts, or when platelet clumping, giant platelets, or interference from non-platelet particles and platelet anomalies are present.[3]

Neubauer manual count is the oldest method of counting platelets and remarkably, is still used as the standard method. However, manual methods are time consuming, subjective and tedious with high levels of imprecision.[4]

Besides this, manual evaluation of the peripheral blood film offers additional information on platelet size, shape, granulation, and the presence of phenomena such as aggregation or platelet satellitism. Platelet estimation through peripheral blood smear (PBS) is simpler and can be effectively used for screening, diagnosis, monitoring disease progression, and assessing therapeutic response.[5,6]

This study was conducted to evaluate platelet counts in patients with thrombocytopenia and thrombocytosis using three methods: automated counting, Neubauer manual counting, and PBS examination.

MATERIALS AND METHODS

A hospital based cross-sectional study was carried out in the Department of Pathology, Nepal Medical College and Teaching Hospital, Attarkhel, Gokarneshwor-8, Kathmandu over a period of six months from July 2024 to December 2024. Ethical clearance was obtained from the Institutional Review Committee of Nepal Medical College. Convenience sampling technique was applied to include samples from the clinical pathology laboratory. Blood samples for complete blood count or platelet count received in the Department of Pathology were enrolled in the study. All cases of thrombocytopenia and thrombocytosis of any age group detected by the automated method were included in this study. Samples not received in EDTA tubes, improperly labelled, hemolysed, clotted and inadequate are excluded. Thrombocytopaenia refers to platelets value less than 150000/µl whereas thrombocytosis refers to platelets value more than 450000/µl (Table 1).

Table 1: Categorization of platelet status based on platelet count[7]

Platelet Status	Cases n (%)	Platelet count
Thrombocytopaenia	246 (76.6%)	<150000/µl
Thrombocytosis	75 (23.4%)	>450000/µl
Total	**321**	

Automated method: Platelet counts were obtained using a hematology analyzer, Sysmex XN-550 (Japan) and principle of measurement is based on flow cytometry. The quality control of the process was monitored as recommended by the manufacturer.[8] After running the sample in automatic analyzer, samples were assessed using Neubauer method and PBS.

Neubauer manual method: It also known as standard method for platelet count. The blood sample was diluted in the ration of 1:20 with a diluting fluid ammonium oxalate (1%w/v) that lyses red blood cells while preserving platelets. The diluted sample was then carefully loaded into the Neubauer chamber and then left the chamber undisturbed for 20 minutes to settle down the platelets. The chamber was examined under a microscope, and the platelets were counted in large center square in 40x objectives. Platelets in all 25 squares within the large center square were counted.

The number of platelets counted in the specific squares were used to calculate the platelet count per microliter of blood using a formula that takes into account the dilution factor and the volume of the counting area.[9]

PBS manual method: For PBS, a drop of sample was carefully placed onto a clean glass slide, where it was spread evenly using another slide to achieve a thin film. After this, the slide was treated with methanol-based fixative, to preserve the cellular morphology. Once fixed, the slide was stained using Wright's stain.

Subsequently, the stained slide was rinsed with a buffer solution to eliminate excess dye and then left to air-dry. Finally, the prepared slide was examined under a microscope with 100x oil immersion lens. The average number of platelets was calculated and was multiplied by fifteen thousand. In an ideal zone of blood film, each platelet on an average 100x oil immersion field represents 15,000 platelets/µl. Samples was analyzed within four hours of collection to minimize platelet clumping.[10]

Descriptive statistics such as frequency, percentage, mean and standard deviation was used to present the data. Data analysis was performed using SPSS-17. ANOVA test was applied to compare mean platelet value among different methods. Pearson's correlation test was applied to compute correlation between methods. P-value less than 0.05 was considered statistically significant.

RESULTS

A total of 321 individuals were included in the study among which 246 were thrombocytopenic and 75 were thrombocytosis case. The individuals were distributed according to age category with mean age 44.3 year, minimum age1 year and maximum age 94 years. In age wise distribution of thrombocytopenia, the frequency of age group between 41-50 years was the highest (20.3%) and in case of thrombocytosis the frequency of age group between 31-40 years was the highest (21.3%) (Table 2).

Table 2: Age wise distribution of cases

Age Group (years)	Thrombocy-topenia (n %)	Thrombocy-tosis (n %)
0-10	2 (0.8%)	11 (14.6%)
11-20	22 (8.9%)	5 (6.6%)
21-30	36 (14.6%)	12 (16%)
31-40	31 (12.6%)	16 (21.3%)
41-50	50 (20.3%)	11 (14.6%)
51-60	48 (19.5%)	8 (10.6%)
61-70	28 (11.3%)	7 (9.3%)
71-80	20 (8.1%)	5 (6.6%)
81-90	8 (3.2%)	-
91-100	1 (0.4%)	-
Total	**246 (100%)**	**75 (100%)**

Out of the total participants, 193 were male and 128 were female. Among males, 79.8% were thrombocytopenic and 20.2% had thrombocytosis. Among females, 72.0% were thrombocytopenic and 28% had thrombocytosis. (Fig. 1).

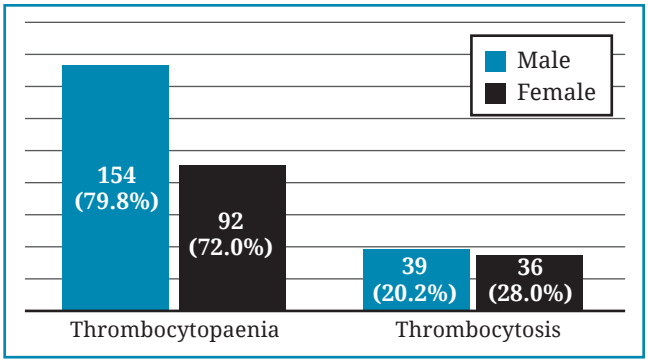

Fig. 1: Distribution of platelet status according to gender

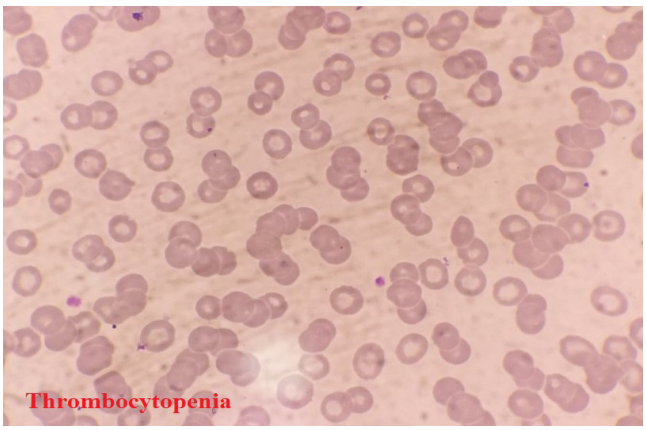

Fig. 2 A: Thrombocytopaenia under 100 X objective lens

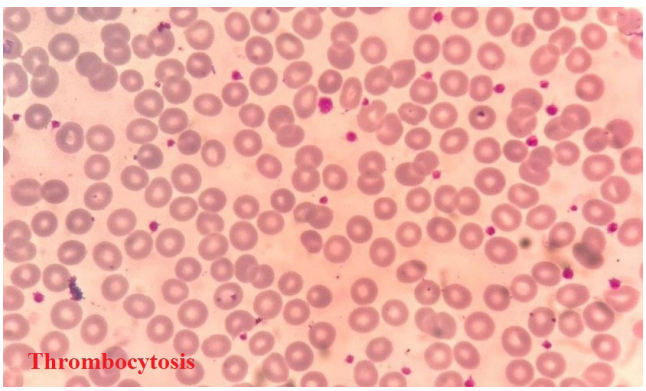

Fig. 2 B: Thrombocytosis under 100 X objective lens

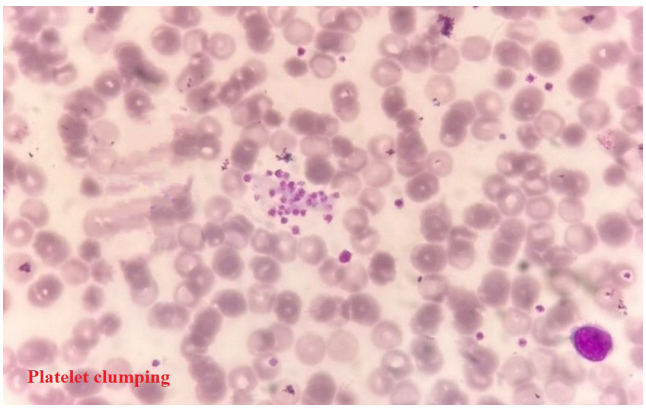

Fig. 2 C: Platelets clump under 100 X objective lens

Table 3: Comparison of platelet status between automated, Neubauer manual and PBS method

Platelet status	Automated (mean ± SD, µl)	Neubauer manual (mean ± SD, µl)	PBS (mean ± SD, µl)	P-value
Thrombocytopenia (n=246)	79,000 ± 33,296	93,900 ± 40,130	91,200 ± 37,560	0.08
Thrombocytosis (n=75)	597,000 ± 148,000	590,000 ± 164,000	601,000 ± 180,000	0.4

The Table 3 shows mean platelet value in case of thrombocytopaenia and thrombocytosis and their comparison between the methods. ANOVA test was applied to compare mean platelet value among three different methods and P value less than 0.05 was considered statistically significant.

The study showed no statistically significant difference in platelet counts measured by the automated, Neubauer manual, and PBS methods for thrombocytopenia and thrombocytosis cases. The percent difference in platelet count in case of thrombocytopaenia between automated and Neubauer manual method is 15.8% while

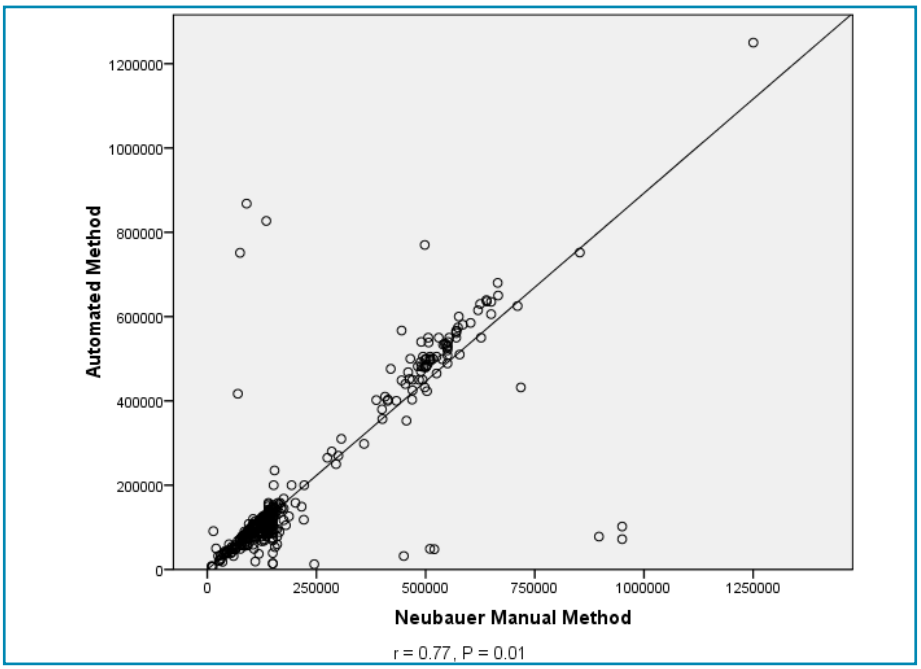

Fig. 3: Correlation between automated and Neubauer counting manual method

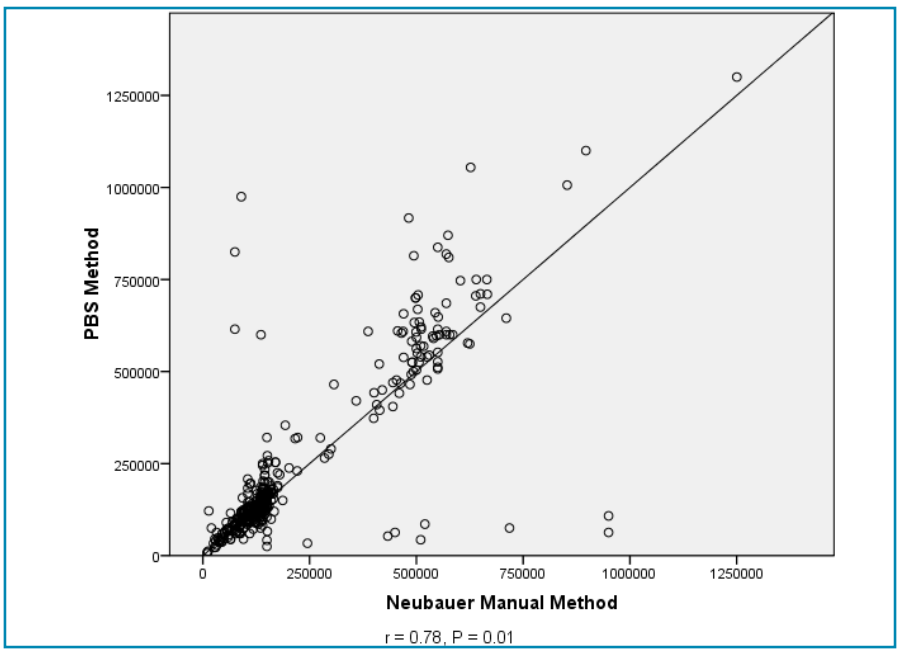

Fig. 4: Correlation between PBS and Neubauer counting manual method

between PBS and Neubauer manual method is only 2.8%. However, the percent difference in platelet count in case of thrombocytosis between the methods is less than 2%.

The scatter plot shows positive correlation (r=0.77, P=0.01) between automated and Neubauer manual platelet count method, with most data points aligning along the diagonal reference line (Fig. 3).

There is positive correlation (r=0.78, P=0.01) between PBS and Neubauer manual platelet count method, with most data points aligning along the diagonal reference line (Fig. 4).

DISCUSSION

The findings of platelet count measured by automated, Neubauer manual, and PBS methods remains challenging till date. The information about level of correlation between methods is limited. In our study, we tried to compare platelet count by three different methods to evaluate their correlation and trends in thrombocytopenia and thrombocytosis.

In this study, out of a total of 321 participants, 193 were male and 128 were female. Among them, 79.8% of the males and 72.0% of the females were thrombocytopaenic, while 20.0% of the males and 28.0% of the females had thrombocytosis. Castromayor *et al*[11] has reported thrombocytopenia affect both male and female sex equally. The age of participants ranged from 1 to 94 years, with a mean age of 44.32±20.1 years, ensuring broad representation of platelet counts across various age groups. This age diversity enhances the generalizability of the study findings, allowing for a better understanding of platelet count variability across the lifespan. Among the different age categories, thrombocytopaenia was most prevalent in the 41–50-year age group (20.3%), while thrombocytosis was most common in the 31–40-year age group (21.3%). Biino *et al*[12] found that thrombocytopaenia is least common in individuals under 18 years of age and most prevalent in those over 80 years. In contrast, thrombocytosis is least frequent in the 60–69-year age group and most common in individuals younger than 18 years. Castromayor *et al*[11] also reported that higher the age group, the higher is the incidence of thrombocytopenia and was most common between 6th to 8th decades of life affecting 36.0% of patients.

In this study, platelet counts were compared across three methods: automated analysis, Neubauer manual method, and PBS examination. The results indicated no statistically significant differences in platelet counts among the three methods for both thrombocytopaenic and thrombocytosis cases. This finding suggests that all three methods are reliable for assessing platelet counts in patients with thrombocytopaenia and thrombocytosis. However, an interesting trend emerged in the thrombocytopenia group. In case of thrombocytopaenia, the automated method tends to report 15.8% lower than standard Neubauer manual method while PBS method reports only 2.8% lower than standard method. This discrepancy may be due to inherent limitations of automated analyzers, such as difficulty in accurately detecting clumped or giant platelets. These findings highlight the potential need for manual confirmation in certain cases. Further studies with larger sample sizes are recommended to validate and generalize these observations. Despite these differences, positive correlations were found among the automated, Neubauer manual, and PBS methods, indicating a high degree of agreement and reinforcing their reliability in platelet count estimation.

In agreement with our findings, Rana *et al*[13] reported a significant positive correlation between the manual method and the automated analyzer. However, this correlation showed limitations at the extremes of platelet counts—particularly in cases of very high or very low values. In thrombocytopaenic patients, notable discrepancies were observed, often due to the presence of platelet clumps, aggregation, or irregular distribution. These findings underscore the importance of careful and thorough platelet assessment, especially in cases requiring precise quantification.

Jain[7] reported a significant positive correlation between the manual slide method and the automated analyzer. This suggests that the manual method can serve as a reliable alternative, particularly in smaller laboratories with lower patient volumes, where the cost of acquiring, operating, and maintaining an automated blood cell counter may be prohibitive.

Similar to our findings, Prajapati[14] also reported no statistically significant difference (p=0.06635) between platelet counts obtained by the manual PBS method—calculated as the average platelet count per high-power field (100x) multiplied by 15,000/µl (207.13±15.898 × 10^3/µl)—and those measured by the automated cell counter (206.53 ± 16.278 × 10^3/µl). Furthermore, a significant positive correlation was observed between the two methods (r=0.9995, p <0.001), as determined by the Pearson correlation test.

Malok *et al*[15] also reported that PBS method provided more agreement with automated thus blood smears is reliable to evaluate automated results and appears to provide adequate quality assurance.

In contrast to our study, Castromayor *et al*[11] found a significant difference between manual and automated platelet count results ($p < 0.05$). Similarly, Rachid *et al*[16] reported that despite good correlations among the various techniques, analyzer-based methods tended to overestimate platelet counts. Notably, the impedance method failed to provide platelet counts in 15.0% of samples, as the analyzer yielded blank results. These particular samples showed low platelet counts ($<15 \times 10^9$/L) when measured by the optical method.

This study concluded that while all three methods generally provide comparable results, the automated method showed a notable tendency to underreport platelet counts in the thrombocytopenic group. The manual method holds its ground, ensuring reliability by addressing concerns like platelet clumping or uneven distribution.

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REFERENCES

1. Campbell NA. Platelets are pinched-off cytoplasmic fragments of specialized bone marrow cells. In: Cain M, Minorsky P, Reece J, Urry L, Wasserman eds. Biology. 8th edn. London: Pearson; 2008.
2. Briggs C, Harrison P, Machin SJ. Continuing developments with the automated platelet count 1. *Int'l J Lab Hematol* 2007; 29: 77-91
3. Tariq A, Rashid A, Khalid H, Latif SA, Ilyas S, Ria MN. Comparison of platelet count by automated and manual methods in thrombocytopenia patients. *J Haematol Stem Cell Res* 2023 12; 3: 17-20.
4. Zhang M, Gu L, Zheng P *et al*. Improvement of cell counting method for Neubauer counting chamber. *J Clin Lab Annals* 2020; 34: e23024.
5. Shafi A, Jeelani N, Jeelani M. Comparison of platelet count by automated and manual methods, a study and review of literature in a medical college hospital in Kashmir. *British J Med Health Sci* 2020; 2: 177-88.
6. Anchinmane VT and Sankhe SV. Utility of peripheral blood smear in platelet count estimation. *Int J Res Med Sci* 2019; 7: 434-7.
7. Jain DK. Comparison of platelet count by manual and automated method. *Int J Res Med Sci* 2020; 8: 3523-7.
8. Sandhaus LM, Osei ES, Agrawal NN, Dillman CA, Meyerson HJ. Platelet counting by the coulter LH 750, sysmex XE 2100, and advia 120: a comparative analysis using the RBC/platelet ratio reference method. *Amer J Clin Pathol* 2002 1; 118: 235-41.
9. Oliveira RA, Takadachi MM, Nonoyama K, Barretto OC. The absolute recommendation of chamber Neubauer method for platelets counting instead of indirect methods in severe thrombocytopenic patients. *J Brasileiro de Patologia e Medicina Laboratorial* 2003; 39: 139-41.
10. Moreno A and Menke D. Assessment of platelet numbers and morphology in the peripheral blood smear. *Clin Lab Med* 2002; 22: 193-213.
11. Castromayor J and Cadete LA. Pb2242 comparison between manual and automated platelet counts of adult patients with thrombocytopenia at st. Paul's hospital iloilo (sphi), philippines. *HemaSphere* 2019; 3: 1005.
12. Biino G, Balduini CL, Casula L *et al*. Analysis of 12,517 inhabitants of a Sardinian geographic isolate reveals that predispositions to thrombocytopenia and thrombocytosis are inherited traits. *Haematologica* 2010; 96: 96.
13. Rana H and Sheikh M. Estimation and comparison of platelet count: automated analyzer vs manual peripheral smear method. *Inter J Adv Res* 2023 ; 11: 621-8 (ISSN 2320-5407)
14. Prajapati AK. Comparison of platelet counts estimated by peripheral blood smear examination and automated haematology analyzer. *Inter J Multidisciplinary Res* 2023; 5: 8.
15. Malok M, Titchener EH, Bridgers C, Lee BY, Bamberg R. Comparison of two platelet count estimation methodologies for peripheral blood smears. *Amer Soc Clin Lab Sci* 2007 1;20:154-60.
16. Mohamed-Rachid B, Raya AF, Sulaiman AH, Salam AK. Comparative analysis of four methods for enumeration of platelet counts in thrombocytopenic patients. *J Applied Hematol* 2015; 6: 119-24.